SOME CONTRIBUTIONS OF MICROBIOLOGY TO CANCER RESEARCH[1]

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A quarter century ago, cancer research was almost exclusively in the domain of the pathologist, the biologist, and the clinician. A cursory examination of the cancer literature at that time reveals that attention was focused predominantly on problems ranging from cytological and histological studies of spontaneous tumors to investigations on homologous and heterologous transplantation of neoplasms, from serological diagnosis of human cancer to immunological studies in fowl sarcoma, from blood chemistry of tumor-bearing hosts to the role of heredity in carcinogenesis. Cancer research was characterized by its intensive rather than extensive orientation; the annual meeting of the pertinent scientific society was held in a single session and was devoted to the presentation of about a dozen papers; many of the problems which occupy the center of the stage today were little more than shadows waiting in the wings.

In the intervening years,the elusive problems of neoplastic growth have been attacked from so many different points of view that today there are few scientific disciplines which have not contributed in some way to its understanding. The number of active investigators devoting their full time to oncology and the diversity of their backgrounds and interests have increased beyond all expectation. At the last annual meeting of the American Association for Cancer Research, there were as many as four concurrent sessions on each of three days, and the scientific program consisted of close to 200 reports. The efforts of cancer researchers have remained intensive, but they have now extended to practically every conceivable aspect of biology and biochemistry.

Microbiology has also been drawn into this development. A few decades ago, even a collector of biological curiosities would have demurred at the suggestion that there was common ground between bacteriology or protozoology and cancer research. Today, microbiological systems constitute a considerable portion of the experimental material in many cancer research centers. In a recent analysis of the financial support of cancer research by government and nongovernment agencies (1), the total expenditure for 1953 was placed at about ten million dollars; at least 7 per cent of these funds was committed for studies which were devoted predominantly to some microbiological system.

Viruses have long been known to cause a variety of neoplastic conditions and are considered by many investigators to be primary causative agents of all cancer. The proponents of the somatic mutation theory, the other principal hypothesis dealing with carcinogenesis, have been interested in the contributions of bacterial genetics and bacterial mutations. Studies on the effect of chemical carcinogens on yeasts, ciliates, and other organisms and on the action of carcinogenic and mutagenic agents on *Hemophilus influenzae* transforming principle are other examples of the application of microbiology to this area of research. Oncolytic viruses, crown-gall tumors, bacteriophage metabolism, elucidation of metabolic pathways with the neurospora and other microorganisms, enzyme adaptation studies in bacteria, and related subjects of current interest are illustrations of the increasingly fruitful union of the two disciplines.

No single short review can begin to deal adequately with these diverse and often complex problems. In selecting a few topics for discussion, the reviewer was guided by one of the more useful definitions of "cancer" which have been coined over the years. In this definition, cancer is considered to involve the uncontrolled growth of cells that have failed to differentiate completely. Most of the investigations in oncology have concerned themselves with the problem of uncontrolled growth; the first section of this review deals with some contributions of microbiology to the search for effective means of stopping the rapid proliferation of the cancer cell. Increasing attention is now being given to the failure of

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differentiation which also characterizes neoplastic cells; the second section of this review is devoted to a consideration of certain slime molds and related organisms which appear to lend themselves particularly well to studies on differentiation and morphogenesis. Some of these studies may have a more tenuous or less immediate connection with the cancer problem than others, but no attempt has been made to apportion space in this review in relation to considerations of this sort.

MICROORGANISMS AND CANCER CHEMOTHERAPY

There are at least four areas of research where microbiology has made significant contributions to cancer chemotherapy. The tumor-inhibitory effects of microorganisms and particularly of some of their products have been widely studied. A number of the more useful chemotherapeutic agents were first discovered in microbiological systems. Studies on the mechanism of action of these agents in bacteria and protozoa have contributed significantly to our present understanding of their anticancer activity. The development of resistance to initially effective chemicals by animal and human leukemic cells, which has been a major obstacle to the realization of the full potential usefulness of the agents we now have, is closely similar to the equally vexing development of resistance to antibiotics by bacteria.

The use of bacteria, fungi, and protozoa and of some of their products as carcinostatic or carcinolytic agents will not be considered here since the literature on this subject has been covered painstakingly and critically in a recent review by Reilly (2; *cf* also 3). The striking parallels between bacterial and neoplastic cell resistance to chemotherapeutic agents have been pointed out by several reviewers (4, 5, 6, 7), and little could be added here to their discussion of the mechanisms underlying the development of resistance. The other two topics, however, will be discussed in more detail.

Discovery of Carcinostatic Agents in Microbiological Studies

While surgery and radiation continue to be the principal tools for the eradication or control of neoplasms, the last decade has witnessed significant progress in chemotherapy, a form of treatment of particular importance in disseminated cancer and in the various forms of leukemia and lymphoma. During this time, thousands of chemical compounds and preparations of the most varied nature have been evaluated in screening programs based on the response of one or several transplanted animal tumors (8). Agents have been selected for testing for many different reasons; these have ranged from the considered conclusions of elaborate studies on the biochemical properties and functions of tumors to the results of unashamed and enthusiastic speculation. From these countless trials there have emerged a handful of compounds which inhibit the growth of experimental tumors or affect the clinical course of human neoplasms without forbidding toxicity to the host.

One of the main concepts which has guided investigators in their search for active agents has been visualized as the "lock-and-key" hypothesis. The development of the antimetabolite theory from the observations of Fildes (9) on the antibacterial action of mercury and of Woods (10) on the relationship between the sulfonamides and *p*-aminobenzoic acid in bacterial metabolism has been amply documented (11). The idea, deceptively simple in retrospect, that an essential biochemical reaction can be blocked by the introduction of an agent which closely resembles the substrate of the reaction and competes successfully with it for the enzyme controlling the reaction, has led to the design and preparation of numerous series of potential inhibitors of bacterial growth. It is of interest that an appreciable number of the effective carcinostatic agents in current use were suggested for screening against tumors on the basis of their demonstrated antibacterial activity and that many of the compounds in this group may be classed as antimetabolites.

The history of the recognition, isolation, and identification of pteroylglutamic acid as an essential growth factor for microorganisms is too well known to bear repetition here. In 1947, the first antimetabolites for pteroylglutamic acid were prepared (12, 13) and shown to inhibit the growth of *Lactobacillus casei* and *Streptococcus faecalis* in a competitive manner. These compounds were promptly employed in the treatment of acute leukemia in children, on the basis of earlier observations of an acceleration of tumor development following the administration of pteroyldi- and triglutamic acid, and found to produce significant though temporary remis-

sions (14). The folic acid antagonists, particularly aminopterin and A-methopterin, continue to be of value in the treatment of leukemias in man and have been shown to inhibit the growth of a great variety of experimental tumors.

The road from demonstration of activity against microorganisms to introduction into cancer chemotherapy has been less direct for another agent of considerable experimental interest, 5-amino-7-hydroxy-1H-v-triazolo(d)-pyrimidine or 8-azaguanine. The gradual elucidation of the central role of nucleic acids in the economy of the cell and of the pathways of their synthesis from simpler precursors had focused increasing attention on the vast array of chemicals which might act as antimetabolites for purines and pyrimidines. In a search for effective purine antagonists, it was found in 1945 (15) that 8-azaguanine, which differs from guanine only by the substitution of a nitrogen atom for the >CH group in position 8, was effective against *Escherichia coli* and *Staphylococcus aureus*; the inhibition was reversed by guanine. This compound was included in the first group of purines which were screened against Sarcoma 180 but failed to inhibit the growth of this tumor (16) and might well have been relegated to the large group of agents which have not been studied further had it not been for the renewed interest occasioned later by its unusually high activity in an entirely different microbiological system.

The animal microorganism, *Tetrahymena geleii*, has long been the subject of extensive investigation from many different points of view (17). Some of the most interesting data have been obtained by a systematic study of the growth requirements of this organism. Following the demonstration that an exogenous source of purine was required for growth, about two dozen substituted purines were tested for their ability to replace or spare guanine or to inhibit growth in a competitive or noncompetitive manner. Among the inhibitors, 8-azaguanine was by far the most effective; the inhibition was completely reversed by guanine (18). Since *Tetrahymena geleii* differed from normal mammalian cells by having this requirement for exogenous guanine and since the possible differences in purine utilization between normal and neoplastic cells were under active investigation at that time, Kidder and his collaborators extended their studies from the animal microorganism to experimental tumors (19). It was found that 8-azaguanine inhibited tumor growth profoundly. These investigators were fortunate in having chosen for this demonstration mammary adenocarcinoma EO771 and two other tumors which responded to 8-azaguanine, since later studies have indicated that experimental neoplasms differ widely in their susceptibility to this agent.

Four other antimetabolites which have proved to be effective in inhibiting the growth of experimental tumors were first suggested for screening on the basis of their activity in *Lactobacillus casei*. These compounds, 2,6-diaminopurine (20), thioguanine (21), 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-ethylpyrimidine (22), and particularly 6-mercaptopurine (21), represent some of the more significant therapeutic results attained by the systematic investigations of Hitchings and his collaborators which will be alluded to in the following section.

The justifiable emphasis placed in these pages on the value to cancer chemotherapy of agents first discovered in microbiological systems should not be construed as an open invitation to the oncologist in search of new agents to rely solely or even predominantly on bacteria, fungi or protozoa for an effective screening method. Many compounds which inhibit a wide variety of microorganisms, including the most potent antibiotics, have failed to exhibit any antitumor activity; effective carcinostatic agents which cannot be considered as antimetabolites have frequently shown no striking antimicrobial activity(23).

Studies on Mechanism of Action of Carcinostatic Agents in Microbiological Systems

The recent review literature abounds with summaries and discussions of nucleic acid metabolism in microorganisms ranging from species of *Lactobacillus* to *Tetrahymena* and *Paramecium* (24–30). In many instances, the biochemical processes in microorganisms have been compared and contrasted with those in mammalian tissues, and the differences and similarities between the two types of biological systems are emerging from these studies. In general, the synthesis and metabolism of the nucleic acids are carried on in a similar way in microbial and mammalian cells; if the specific differences between particular systems are clearly understood (29), much of the information gained in microorganisms can be a guide for

the study of normal and neoplastic mammalian cells.

In this section, no attempt will be made to present a definitive résumé of this rapidly developing field. Rather, it is intended to illustrate the importance of this sort of investigation by citing a few specific reports dealing with the action of carcinostatic antimetabolites in microbiological systems.

In the past decade or so, a comprehensive program of the biosynthesis of nucleic acid derivatives in *Lactobacillus casei* has been developed by Hitchings and his collaborators. An impressive number of purines, pyrimidines, pteridines and related compounds have been prepared and tested in the expectation that the discovery of inhibitors would lead to the elucidation of the biochemical pathways involved in nucleic acid synthesis and that this program would yield new chemotherapeutic agents for bacterial, rickettsial, viral, protozoal or neoplastic diseases (31). These expectations have been amply fulfilled; in addition to the important contributions to cancer chemotherapy mentioned earlier, this program has caused significant advances in the chemotherapy of other diseases, particularly malaria. The *Lactobacillus casei* model has served, moreover, to shed some light on the mechanism of action of antimetabolites related to folic and folinic acids and to nucleic acid derivatives. A brief consideration of this model system may therefore be in order.

The organism was grown on a basal medium deficient in purines, uracil, and folic acid and on six variants of this medium achieved by the addition of a small or an excess amount of folic acid, adenine, thymine, adenine + thymine, or adenine + folic acid. The response of the bacteria on each of these media to the compounds under investigation permitted a rapid definition of their mechanism of action in this system in terms of their capacity to replace folic acid, thymine, or purine or to inhibit biochemical reactions for which one of these entities was the substrate. On the basis of these data, the 2,4-diaminopyrimidines including the antimalarial Daraprim and its carcinostatic dichloro analog have been classed as powerful antagonists of folic or folinic acid, a conclusion which has been confirmed in mammals (32); 6-mercaptopurine has been shown to be a purine antagonist in the bacterial system (21) as well as in experimental neoplasms (33).

Several other studies have employed the same technique of inhibition analysis, in which the biochemical site of action of an inhibitor is defined in a more or less general way by the identity of a normal metabolite which prevents or reverses its inhibitory action. A brief listing of results in microbiological systems and in a few types of experimental tumors was presented in the recent review by Skipper (4) which gave an incisive summary of the evidence for the attractive hypothesis that the mechanism of carcinostasis primarily involves the nucleic acids of the cancer cell.

It has recently been realized that an antimetabolite may not only interfere with the utilization of the normal metabolite for a particular synthetic pathway but may itself be utilized in its place, leading to the formation of unnatural end products of that pathway which may be thought of as antimetabolites at that more complex level. In nucleic acid biosynthesis, the present cynosure of cancer chemotherapy, an antipurine might thus be incorporated in place of the purine to form a nucleic acid molecule which would interfere with mitosis or fail to be attached properly to protein to form nucleoprotein or be incapable of reaching the correct degree of polymerization. An example of this type of reaction is the incorporation of 8-azaguanine into nucleic acid in tumor-bearing mice (34, 35), in *Tetrahymena geleii* (36, 37), and in tobacco mosaic virus (38).

It is, of course, tempting to postulate a causal relationship between incorporation of the antimetabolite and inhibition of growth of the neoplastic cell, particularly in view of the finding that the incorporation of 8-azaguanine into the ribonucleic acid of an azaguanine-susceptible strain of leukemia was much greater than into the ribonucleic acid of an azaguanine-dependent variant (39). However, in resisting this temptation at the present time the investigator is armed not only with his natural reluctance to generalize from a single well substantiated illustration to a general explanation of the growth inhibition, but also with recent data on the incorporation of 5-bromouracil into nucleic acid of *Streptococcus faecalis* and *Enterococcus stei* (40, 41) and of *Escherichia coli* (42, 43), data that clearly indicate that the incorporation of this thymine antagonist may be as great in cells whose growth is not inhibited by this compound as in cells which respond to it. The full significance of information from this type of experiment is still under active investigation.

This section may be concluded with a brief mention of the interesting experiments of Stock and collaborators on the effect of 2,6-diaminopurine on kappa in *Paramecium aurelia*. This work, which was recently reviewed by these investigators (44), illustrates the wealth of material which can be made available by judicious selection of a microbiological system for the elucidation of the mechanism of action of carcinostatic agents.

Recapitulation

As ontogeny recapitulates phylogeny, so the developmental history of some of the recent agents of chemotherapeutic interest shares the characteristic features which have been outlined in these pages. This is particularly well illustrated by O-diazoacetyl-L-serine, or azaserine, one of the newest additions to the armamentarium of experimental cancer chemotherapy.

This agent was isolated from laboratory broth cultures of a species of *Streptomyces* (45) and was found to inhibit the growth of Sarcoma 180 (46) and other neoplasms (47). It has shown antibiotic activity against a variety of microorganisms (45, 48), one of which has been used for the assay of activity during isolation. Studies on the mechanism of action of azaserine in *Escherichia coli* (49, 50) suggest that it may inhibit amino acid synthesis by interfering with the condensation of indole and serine to form tryptophan and with the formation of tyrosine and phenylalanine from their precursors. Analagous studies in tumors have not been reported; the only data available in neoplastic tissues implicate formate incorporation into nucleic acids as a site of inhibition by azaserine (51). The development of an azaserine-resistant line of mouse leukemia has been reported (47).

It should not be inferred from this discussion that all research in cancer chemotherapy is moving along these paths. In this active field, many approaches are being utilized, and microbiology is only one of the numerous disciplines which are contributing ideas and leads. Within this framework, however, it can be concluded that it is one of the most rewarding areas of investigation.

MICROORGANISMS AND DIFFERENTIATION

The definition of "cancer" which was cited earlier bears witness to the fact that the failure of neoplastic cells to differentiate is as important to an understanding of this disease as their capacity for unrestrained growth. The more malignant tumors are endowed not only with greater invasiveness and a higher rate of proliferation but frequently also with a greater degree of anaplasia which causes them to have little resemblance to the highly differentiated tissues from which they are derived.

The term "differentiation" is widely used, but its meaning is rarely spelled out. For the present discussion, Needham's (52) definition may be the most serviceable: "Increase in complexity and organization. Increase in number of kinds of cells; invisibly, as by determination and losses of competence; visibly, as by histogenesis. Increase in morphological heterogeneity by the appearance of form and pattern."

Despite the abstract acceptance of the need to understand the mechanisms of differentiation as clearly as the causes of uncontrolled growth, most experimental inquiries in oncology have addressed themselves to the latter aspect of the problem. There are many reasons for this. Our knowledge of synthetic processes in biochemistry is much more extensive than our understanding of the more nebulous events which take place in organization and morphogenesis. The rapid multiplication of tumor cells is more easily observed and analyzed than the subtler defects which cause the cells to lose their highly differentiated character. The analytical approach, which to a biochemist usually holds the greatest promise for the elucidation of a problem, has already been used extensively in studying proliferation, whereas most of the work on differentiation has been descriptive. The range of biological systems available for morphogenetic studies is more restricted than the biological material which lends itself readily to experiments on proliferation. This last difficulty, which may be an underlying or at least contributory cause of the other obstacles to progress in this area, is beginning to engage the attention of some experimental oncologists.

Practically all investigations concerned with differentiation have been carried out in embryological systems, from the sea urchin to man. The solid progress in chemical embryology is attested to by the monumental treatises of Needham (52, 53) and Brachet (54) as well as by many recent discussions (55, 56). Two fundamental difficulties are inherent in this type of biological

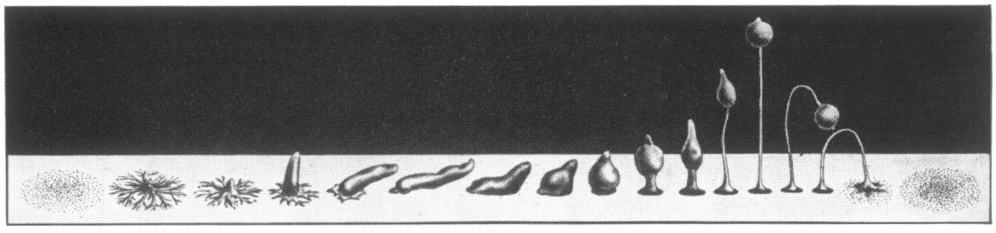

Figure 1. The life cycle of *Dictyostelium discoideum*, from a drawing by Mr. James Lewicki reproduced in *Life* Magazine of April 10, 1950. Reproduced by permission of copyright owner.

material, as repeatedly stressed by Needham: the lack of any quantitative measure of differentiation and the simultaneous occurrence of growth and differentiation at all stages of embryonic life. The analytical approach to the problem of mechanisms of differentiation is seriously hampered by these difficulties; the desirability of studying biochemical changes caused by morphogenesis in the absence of cell proliferation is self-evident.

These considerations prompted a search for biological materials in which differentiation might be investigated in the absence of cell division or at least under conditions where its role is relatively insignificant. Attention was focused on microbiological systems, particularly certain protozoa. At the present time, the value of these systems for the eventual elucidation of the mechanisms of differentiation cannot be assessed with assurance. They are discussed here in the hope that more widespread interest in this approach may speed the day when such an assessment can be made.

Dictyostelium discoideum

This organism and its close relatives among the amoeboid slime molds, or Acrasieae, have long intrigued biologists with their life cycle and behavior patterns. In the past half dozen years, several reviews of experimental studies with these organisms have appeared (57–59), and *Dictyostelium discoideum* has even been singled out for presentation to the general scientific fraternity (60, 61) and to the lay public (62, 63). The aspect which has fascinated a number of observers and which led to the selection of this organism for an examination of the mechanisms of differentiation is the division of its life cycle into two distinct parts. In the first part, the organism is unicellular and carries on active feeding and proliferation; in the second, it becomes multicellular and exhibits a complex series of morphogenetic movements in the virtual absence of cell division.

A detailed description of the life cycle of *Dictyostelium discoideum* may be dispensed with since it has been presented repeatedly (57, 58). Briefly, the following stages are observed (figure 1): (a) germination of spores to liberate myxamoebae; (b) growth and proliferation of myxamoebae, which feed on their bacterial associates; (c) aggregation of the previously independent myxamoebae towards a center, by chemotaxis, to form a pseudoplasmodium; (d) migration of this mass over the substratum; and (e) culmination to form a mature structure, which consists of a basal disk, a thin stalk, and a sorus containing the spores that are ready to release new myxamoebae. The rapidly expanding literature on this organism has concerned itself with a number of problems that will be discussed here briefly, with emphasis on the more recent contributions in this field.

In connection with the elegant series of investigations by Raper and Bonner on the life cycle, growth, and organization of *Dictyostelium discoideum* (57), attention was drawn repeatedly to the obvious desirability of achieving pure culture. In nature, the organism grows on decaying leaves or in the soil, and it was quickly established that live bacteria provide the proper physical and nutritional conditions for its development. In the laboratory, it has been possible to grow it routinely in association with a single bacterial species (57, 64); this has been the method of choice in most subsequent studies. Numerous attempts have been made to replace the living bacteria with a variety of supplements (65, 66), and although routine large-scale pure culture of the slime mold has not yet been achieved, solid progress is being made in this direction. Raper (57) has reported successful growth and development of the slime mold on lactose-peptone agar streaked with *Escherichia coli* killed by sterilization for 20 minutes at 121°C. More recently,

Bradley and Sussman (67) obtained some growth (10–20 per cent of normal) on a sterile medium enriched with a fraction of *Aerobacter aerogenes* which after isolation in homogeneous form has now been characterized as a protein of molecular weight between 25,000 and 40,000 containing the usual spectrum of amino acids (68).

Experiments on the proliferative stage of this organism have been postponed until pure culture methods become available. In the meantime, however, the demonstration by Bonner (58) that the bacteria on which myxamoebae feed can be removed by washing and differential centrifugation and that the myxamoebae will go through the remainder of the life cycle (aggregation, migration, and culmination) in the absence of the bacterial associates has made it possible to study the various stages of differentiation in essentially pure culture.

Bonner has been particularly interested in the control of the morphogenetic movements in *Dictyostelium discoideum* (58, 69, 70, 71, 72). He has demonstrated that aggregation of the independent myxamoebae is brought about by a chemotactic principle, called acrasin, which continues to be emitted by the cells even through pseudoplasmodium formation, migration, and culmination. In the migrating pseudoplasmodium the anterior region, which directs the orientation and movement of the sausage-shaped structure towards light and heat, produces most of the acrasin, and during culmination the capacity to produce this chemotactic principle is retained only by the cells located at the tip of the fruiting structure. Isolation of acrasin for eventual chemical identification has been attempted repeatedly (73), but the identity of this agent, which plays a central role in differentiation in this organism, remains one of the challenging mysteries in this field.

Raper and Fennell (74) have carried out a detailed investigation of stalk formation during culmination of the slime mold. Particular attention was given to the nature of the outer sheath surrounding a column of vacuolated cells in the stalk. This sheath is formed by the extracellular deposition of cellulose in a critical circular zone, the dimensions of which are proportional to the mass of cooperating myxamoebae.

An important contribution has been made by Sussman in a series of reports over the last three years (59, 75, 76, 77, 78, 79). It was realized that the complex pattern of differentiation in this organism could be brought about by the fortuitous association of two or more genetically heterogeneous parent spores or could proceed from a single spore. If the first alternative applies, these studies would remain a fascinating biological occupation, but the results would have general significance for problems in differentiation only if the entire development could start with a single spore. In a series of ingenious experiments, it was shown: (a) that single spores of *Dictyostelium discoideum* and two related species were indeed capable of initiating the normal course of development; (b) that vegetative myxamoebae retained this ability; (c) that at the onset of aggregation only a small proportion of the cells exhibited the capacity of initiating a center; (d) that, however, the ability of the myxamoebae to produce acrasin was not restricted to the "initiator" cells. Irradiation of spores or myxamoebae of the wild type *Dictyosteliaceae* produced a number of variants which failed to aggregate or to produce fruiting structures or which had an abnormal appearance. The importance of correlating these abnormalities with changes in the enzyme pattern of these cells was emphasized, but this as well as the problem of relating them to genic mutations has not yet been approached experimentally. Recent studies on the synergism and antagonism between morphogenetically deficient variants may provide a key to the elucidation of the metabolites and biosynthetic pathways involved in the morphogenetic cycle.

Another problem which has recently received attention relates to the occurrence and role of cell division during differentiation. One of the principal attractions of *Dictyostelium discoideum* for a variety of studies, an attraction noted by all early observers, has been the apparent complete separation of proliferation and differentiation in the life cycle. The general conviction that all mitotic activity ceased before aggregation, however, was recently shaken by the findings of Wilson (80, 81) and Bonner and Frascella (82). Wilson, using smears of *Dictyostelium discoideum* in association with *Escherichia coli*, reported evidence of gamete fusion just prior to, and of meiosis during, aggregation and of a wave of mitoses at the time of spore formation at the end of culmination. Bonner, in experiments on myxamoebae freed of bacteria by washing and differential centrifugation, demonstrated the presence

of mitosis in the early migration stage but found no cell divisions during aggregation or early culmination. It was suggested (82) that differentiation is not dependent on these mitoses, but further work is clearly indicated before the role of cell divisions in early migration and in spore formation is clarified. Some of this new information appears to be well founded, but Wilson's claim of zygote formation prior to aggregation and, in general, his postulate of sexuality in the development of this organism have recently come under concentrated attack based on a number of cogent arguments (59). Until more convincing evidence for sexuality is presented, these claims must be considered at least somewhat speculative.

The application of biochemical techniques to the problems of differentiation in *Dictyostelium discoideum* promises to contribute valuable information on the underlying mechanisms. Work has begun along two general lines, though it must be stressed that only a small beginning has been made. Gregg (83a), studying oxygen utilization during growth and differentiation, showed that morphogenesis cannot proceed under anaerobiosis and that oxygen consumption increases during the transition from the growth phase to the morphogenetic phases, remains constant through the middle of the culmination phase, and then declines to negligible levels. The same author (83b,c) has recently studied the carbohydrate and nitrogen metabolism of this organism during growth and morphogenesis and has demonstrated by ingenious methods that protein is utilized to obtain energy during culmination and to satisfy other morphogenetic requests. Further, comparative analyses of cells at different stages of the life cycle for various biochemical entities have been forecast by preliminary data (66) that demonstrated a decreased content of ribonucleic acid per mg nitrogen and a higher acid-soluble fraction of the total phosphorus in pseudoplasmodia than in preaggregation myxamoebae. More extensive work on these subjects is in progress.

A different, more indirect biochemical approach consists in the inhibition of aggregation or culmination by compounds whose general mechanism of action is known or can be further studied in this test system (84, 85, 86, 87). For most of these experiments, myxamoebae were harvested before aggregation, washed free of bacteria, and allowed to aggregate in standard salt solution containing different concentrations of a potential inhibitor. Figures 2 and 3 provide two views of normal aggregation patterns. In experiments on the inhibition of culmination, aggregation was allowed to proceed, and an agar disk bearing the

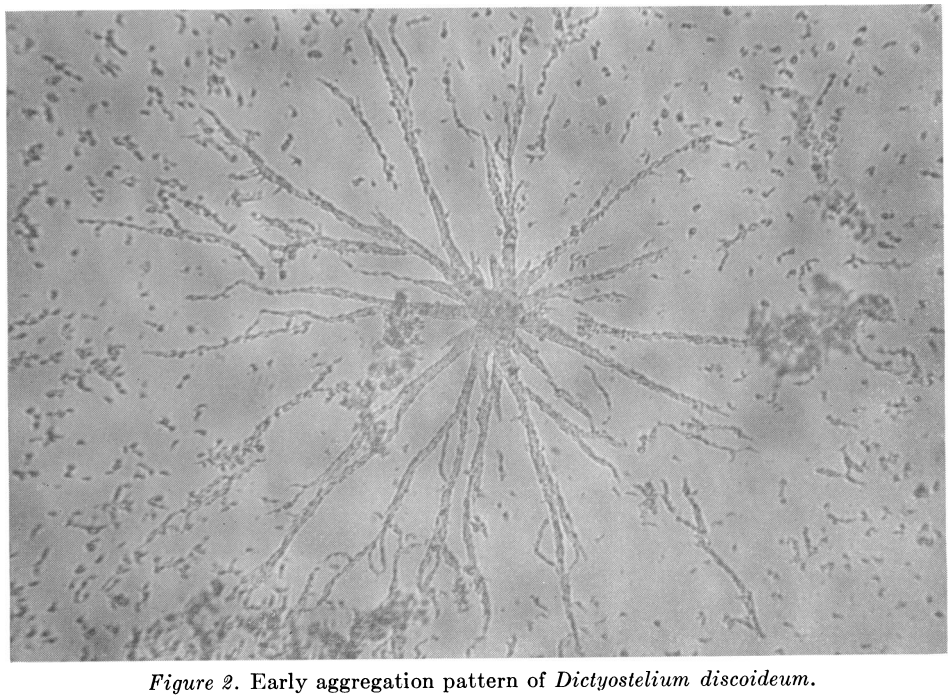

Figure 2. Early aggregation pattern of *Dictyostelium discoideum.*

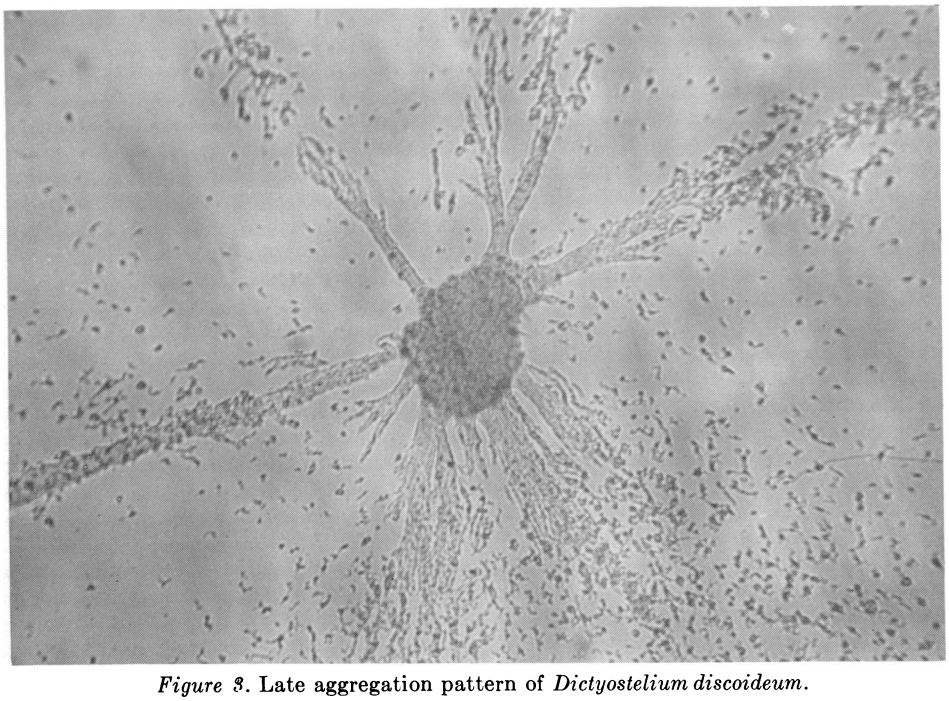

Figure 3. Late aggregation pattern of *Dictyostelium discoideum*.

aggregates was placed in the salt solution with and without the inhibitor. Aggregation was inhibited by compounds of varied biochemical action, including 2,4-dinitrophenol, iodoacetamide, diethylstilbestrol, several azo dyes, and a series of 2,4-diaminopyrimidines, whereas common inhibitors of glycolysis and of mitosis generally had no effect. In most instances, the inhibition of aggregation by these agents was not a simple irreversible process; if myxamoebae were exposed to the inhibitor for several hours and then washed with salt solution, they could aggregate normally. The length of the period of permissible exposure to the inhibitor varied from agent to agent. A more detailed study of the effect of 2,4-dinitrophenol suggested that high-energy phosphate is necessary for aggregation and that inhibition of this process relates to the hydrolysis of organic phosphate to yield increasing amounts of inorganic phosphate. Culmination was more easily inhibited than aggregation under the conditions of these tests, and the differences in response of these two stages of morphogenesis to certain agents provide intriguing opportunities for further investigation.

Although *Dictyostelium discoideum* has been the organism used most extensively in these studies since its isolation by Raper in 1935, other members of the Dictyosteliaceae have also received some attention (88), with generally analogous results.

Myxomycetes and Myxobacteria

The biological literature of the past half century is a treasure house filled with other unusual systems for investigations into differentiation and morphogenesis. The fascinating account of morphogenesis in a wide variety of biological systems by Bonner (58) has done much to stimulate and direct thinking in this field. Reference should also be made to the work of Weisz (89, 90), who developed a general statement on the mechanism of differentiation based on studies in ciliates but of tentative applicability to multicellular organisms as well. The present discussion has been devoted largely to the Acrasieae, because of the numerous recent contributions dealing with this group. This section may be concluded by brief mention of two types of organisms which carry on morphogenesis in a manner similar to that which has been described.

The myxomycetes or true slime molds differ from the Acrasieae in that they have a true plasmodium rather than the multicellular aggregate

of myxamoebae which in *Dictyostelium* and related species is properly called a pseudoplasmodium. A number of these organisms, notably *Physarum polycephalum*, *Physarella oblonga*, and *Fuligo septica*, have been examined from the point of view of nutrition, physiology, taxonomy, and morphogenesis (58, 91, 92, 93, 94, 95). *Physarum polycephalum* has long been a favorite for studies on protoplasmic streaming and chemotaxis (96–101). Many of the problems that have been encountered and the sort of data which have been obtained are similar to the information available in the Acrasieae, but less thought has perhaps been given to the possible applications of these results to general mechanisms of differentiation. The recent demonstration of the suitability of myxomycetes for biochemical studies (102, 103) and the current advances towards pure culture of the plasmodium (H. P. Rusch, unpublished results) greatly increase the attractiveness of these organisms for such investigations.

Finally, brief mention may be made of the myxobacteria, which exhibit striking similarities to the Acrasieae though they are totally unrelated to them from the point of view of taxonomy. These similarities have been emphasized by most observers (58, 104, 105, 106), particularly with regard to the aggregation patterns and, in the higher myxobacteria, the formation of elaborate fruiting bodies. The application to these organisms of the investigative techniques which have been and are being directed at the Acrasieae appears to be one of the most promising tasks for the immediate future.

It was stated at the beginning of this section that the value of these studies to experimental oncology cannot yet be assessed. Results in these microbiological systems have correlated reasonably well with findings in multicellular organisms where such a comparison has been possible; the basic biochemical equipment of these organisms is generally the same as that of mammalian cells. Nevertheless, even the more enthusiastic advocates of these fascinating microorganisms will defer final judgment on the applicability of these systems to an explanation of the failure of differentiation in neoplasia until a good deal of further evidence can be assembled.

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